Metabolic and cellular responses of Atlantic salmon (*Salmo salar*) gill to intermittent hypoxia

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METABOLIC AND CELLULAR RESPONSES OF ATLANTIC SALMON (*SALMO SALAR*) GILL TO INTERMITTENT HYPOXIA

by

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ABSTRACT

Lush, L., Murray, H.M., Hobbs, K., Burt, K., Austin, C., Pretty, J., Whittle, J., Penney, S., Hamoutene, D. 2012. Metabolic and cellular responses of Atlantic salmon (*Salmo salar*) gill to intermittent hypoxia. Can. Tech. Rep. Fish. Aquat. Sci. 2972: v + 15 p.

Juvenile Atlantic salmon were exposed to intermittent hypoxic conditions (4.0 - 5.9 mg L^{-1} dissolved oxygen) over a 47 day period. Fish were killed 18 - 36 hours post exposure and gills excised. Gill tissue from hypoxic and normoxic exposed fish were analyzed for enzyme activity (lactate dehydrogenase, lactate, citrate synthase, cytochrome c oxidase), protein concentrations, and analyzed histologically for abnormalities and eosinophilic granulocyte (EGC) distribution and quantification. Results revealed no differences in any variables tested with the exception of significantly increased protein levels ($p < 0.05$) in the gills of intermittent hypoxia exposed fish. Increased protein levels may likely be an effect of the hypoxia stress response and might signify stress related protein increases in the recovery phase of hypoxia. Eosinophilic granulocytes were found throughout gills of all groups, regardless of environmental stress (hypoxia) confirming results found by some authors suggesting that EGC migration normally occurs within the microcirculation of the gill and does not appear to arise as an immuno-response to environmental stressors as proposed by other authors.

RÉSUMÉ

Lush, L., Murray, H.M., Hobbs, K., Burt, K., Austin, C., Pretty, J., Whittle, J., Penney, S., Hamoutene, D. 2012. Metabolic and cellular responses of Atlantic salmon (*Salmo salar*) gill to intermittent hypoxia. Can. Tech. Rep. Fish. Aquat. Sci. 2972: v + 15 p. [en anglais seulement]

Des saumons atlantiques juvéniles ont été exposés à des milieux hypoxiques de façon intermittente (dont la teneur en oxygène dissous se situait entre 4 et 5,9 mg L^{-1}) pendant 47 jours. Les poissons ont été tués entre 18 et 36 heures après l'exposition et les branchies ont été enlevées. Le tissu des branchies de poissons exposés à des milieux hypoxiques et normoxiques a été analysé pour mettre en évidence les sources d'enzymes (lacticodéshydrogénase, lactate, citrate synthase, cytochrome c oxidase) et les concentrations de protéines; un examen histologique des anomalies et de la distribution et de la quantification des granulocytes éosinophiles a également été fait. Les résultats des analyses n'ont révélé aucune différence dans les variables testées à l'exception d'une augmentation importante des niveaux de protéines ($p < 0,05$) dans les branchies des poissons exposés de façon intermittente à l'hypoxie. L'augmentation des niveaux de protéine peut vraisemblablement avoir été causée par une réaction de stress à l'hypoxie et peut indiquer une augmentation des protéines causée par le stress pendant la phase de rétablissement de l'hypoxie. Des granulocytes éosinophiles ont été décelés dans les branchies de tous les groupes de poissons, soumis ou non au stress environnemental (hypoxie), confirmant ainsi les résultats d'autres auteurs qui proposent que les granulocytes éosinophiles migrent normalement dans le système de microcirculation des branchies et ne semblent pas constituer une réponse immunologique aux stresseurs environnementaux, comme le proposent d'autres auteurs.

INTRODUCTION

Sea cage Atlantic salmon farming in Newfoundland is expanding rapidly, with notable increases in activity on the south coast including Fortune and Placentia bays. In marine cages, levels of dissolved oxygen (DO) are often unpredictable, and common occurrences of intermittent hypoxic conditions have been described by Mansour et al. (2008) and Burt et al. (2011). Such changes may affect the physiology of the fish, as adequate DO is essential to fish health and successful production (Kutty and Saunders 1973; Brett and Groves 1979).

Hypoxia can be defined as the level of DO in which fish make physiological adjustments to maintain an appropriate oxygen level in tissues (Farrell and Richards 2009; Burt et al. 2011). The level of dissolved oxygen at which these changes occur is variable, and differs amongst species, habitat and life stage (i.e., DeSilva and Tytler 1973; Rabalais and Turner 2001; Farrell and Richards 2009). Metabolic and molecular responses to hypoxia are critical to enhance survival at O_2 below adequate levels. For Atlantic salmon, oxygen levels below the critical point of 6 mg L^{-1} (i.e., 66% O_2 at 10°C/34ppt salinity) are considered hypoxic (Davis 1975; DFO 2005; Mansour et al. 2008). Furthermore, it has been suggested that dissolved oxygen levels lower than 6 mg L^{-1} may cause chronic stress and are insufficient to support optimal fish growth (Brett and Groves 1979; Neill and Bryan 1991). Under hypoxic conditions, fish may resort to reducing their metabolic rate or shifting to anaerobic metabolism in order to survive (Cooper et al. 2002). Physiological reactions of some fish species to hypoxia include increased rate of water flow onto the gills to compensate for lack of environmental oxygen, altered blood circulation through the gills to enhance oxygen capacity, and a decrease in phosphate levels in the red blood cells resulting in an increase in haemoglobin (Randall 1982). There is also evidence for an increase in anaerobic metabolism and increased anaerobic enzymes (Randall 1982; van den Thillart and van Waarde 1985).

Aerobic metabolism can be estimated by enzyme activity of cytochrome c oxidase (CCO) and citrate synthase (CS). Measuring the activity of CCO in gills, allows the overall energy produced in the mitochondria to be determined. Reduced enzyme activity is indicative of reduced metabolic rate, reduced oxygen consumption and an indication of exposure to hypoxia (Webb et al. 2005). Citrate synthase is an indicator enzyme of aerobic respiration and exhibits a decrease in activity as oxygen concentration decreases (Cooper et al. 2002).

When oxygen falls below the critical level and fish are stressed, anaerobic pathways can compensate, at least in part, for the reduced energy provision by aerobic metabolism (Virani and Rees 2000). Anaerobic metabolism can be estimated by the activity of lactate dehydrogenase (LDH) and lactate (Ip et al. 1990; Cooper et al. 2002; Webb et al. 2005). Lactate dehydrogenase activity increases under low O_2 levels (Cooper et al. 2002). Lactate is exhibited under

hypoxic conditions, when fish tend to use anaerobic respiration (Zhou et al. 2000; Cooper et al. 2002).

Low O_2 levels appear to result in suppression of the general immune function in fish (Breitburg et al. 2009) and localized inflammation response in gill tissue (Holland and Rowley 1998) due to stress. Burt et al. (in press) reported decreased respiratory burst (RB) activity in head kidney (HK) leukocytes of Atlantic salmon exposed to intermittent hypoxia and proposed that this may increase their susceptibility to disease. Eosinophilic granulocytes (EGCs), a component of the immune system of fish, are often found in increased numbers in chronically inflamed or stressed tissues (Holland and Rowley 1998; Reite and Evensen 2006). In numerous species, accumulation of EGCs has been noted in response to parasites, infections, and tagging lesions (Roberts et al. 1973; Blackstock and Pickering 1980; Poppe and Breck 1997). Powell et al. (1990) noted EGCs in the gills of rainbow trout (*Oncorhynchus mykiss*); however found no evidence to support their presence in response to infection or osmotic stress. Murray et al. (2003) and Murray et al. (2007), showed a population of EGCs in normal winter flounder gill that expressed the anti microbial peptide (AMP) pleurocidin. In addition, other structural changes in the gills of carp, *Cyprinus carpio* (Mustafa et al. 2011), channel catfish, *Ictalurus punctatus* (Scott and Rogers 1980) and sea bass, *Dicentrarchus labrax* (Rinaldia et al. 2005) have been noted in response to sub-lethal levels of hypoxia, including such changes as epithelial lifting, aneurysm, and secondary lamellae fusion.

In this study, we exposed salmon to intermittent hypoxic conditions mimicking conditions recorded on cage sites by Burt et al. (2011). Metabolic enzyme analyses of CCO, LDH, CS, lactate, and protein were performed on gill samples to determine the impact of intermittent hypoxia. Additionally, histological examinations of gill samples were evaluated to determine any structural or cellular differences and abnormalities.

MATERIALS AND METHODS

Cultured male and female juvenile Atlantic salmon were obtained from an aquaculture site on the south coast of Newfoundland and transferred to the Northwest Atlantic Fisheries Center in St. John's, Newfoundland. Groups of 11 fish (222.07 ± 44.82g mean wet mass) were randomly assigned to four 273 L tanks to mimic the stocking density (~9 kg m^{-3}) seen in cages (as described in Burt et al. 2011). Water volumes in tanks were 250 L and maintained at a flow rate of 23 L min^{-1} (water consumption 10 L kg^{-1} fish min^{-1}). Fish were acclimated in tanks for eight weeks before beginning the experiment.

EXPERIMENTAL DESIGN

Four tanks were randomly divided into two experimental groups: normoxia (N) and hypoxia (H). Oxygen levels (mg L^{-1} and % saturation) and temperature were monitored daily with an Oxyguard Polaris handheld oxygen meter (Point Four Systems, Coquitlam, BC, Canada). Mean temperature during the experiment was 9.3 ± 1.9 °C and DO in all tanks was kept at 9.2 ± 2.5 mg L^{-1} (94.6 ± 3.5 % saturation) except when exposed to hypoxic conditions (DO: 4.0 - 5.9 mg L^{-1}; ~44 – 65 % saturation). During hypoxic events, the oxygen in the H treatment was lowered by bubbling nitrogen gas through an air stone (with minimal disturbance to fish) to reach dissolved oxygen levels between 4.0 and 5.9 mg L^{-1} for 30, 60, 90, 120 or 150 minutes. In addition, the N group was also exposed to sham conditions with the addition of an air stone bubbling air instead of nitrogen, to account for any impact the presence of the air stone would have on the treatment groups. In cages on the south coast of Newfoundland, hypoxic DO ranges between 4.68 to 5.99 mg L^{-1} (55.6 – 73.9 % saturation; 16.07 ± 0.64 °C) while length of hypoxic events range from 30 minutes up to 390 minutes (average duration 69.75 min) (Mansour et al. 2008; Burt et al. 2011). In order to mimic the field conditions in a tank based setting, these described conditions were used, and the number and duration of hypoxic events per day were randomly assigned in order to adequately imitate the conditions previously described. The experiment ran for 47 days with intermittent hypoxic events occurring on a total of 38 days with a total of 133 events over the period. The average exposure time was 69.69 min. (30 min. = 39 events, 60 min. = 44 events, 90 min. = 28 events, 120 min. = 12 events, 150 min. = 10 events).

During the experiment, the N and H fish were manually fed Optiline 3 mm commercial pellets (Skretting, NB, Canada) twice daily at 2% wet body mass (BM) until apparent satiation was reached. Satiation was determined as the point when fish stopped actively feeding and pellets remained on the bottom of the tanks for 5 minutes. Any remaining pellets were removed from the tank following feeding.

SAMPLING

At the end of the experiment (day 47), 10 fish from each tank were euthanized with an overdose of TMS (n=20 per treatment). Sampling occurred at 13 hours post hypoxia exposure for half the tanks and 37 hours post exposure for the remainder. It was necessary to space the sampling in this manner in order to gain access to time sensitive equipment for other aspects of this hypoxia study (Burt et al. in press). Gills were excised from left/right gill arch. Several filaments were placed in plastic weigh boats on dry ice while the remainder of the gill was fixed in 10% buffered formalin.

ENZYMATIC ANALYSES

Gills enzyme activity including lactate dehydrogenase (LDH), citrate synthase (CS), cytochrome c oxidase (CCO), lactate, and protein were analyzed. Enzymatic analyses were carried out for 7 samples from each tank (n=14 per treatment). Before analysis, the gills were prepared according to S9 protocol (Veinott et al. 2003). More specifically, approximately 1g of sample was weighed and a volume of TRIS-buffer four times the sample weight was added. Samples were homogenized with a Polytron PT 3000 homogenzier, placed in Eppendorf tubes and frozen at -70°C until analyzed. Lactate dehydrogenase was determined using the method described in Mitchell et al. (1980). Citrate synthase and CCO were measured with commercial kits: SIGMA CS0720 and SIGMA CYTOCOX1 respectively. Lactate was determined by a colorimetric rate determination method using the Abcam Lactate Colorimetric Assay Kit ab65331, and protein analysis was conducted according to Lowry et al. (1951).

GILL HISTOLOGY

Gills from three fish in each tank (n=6 per treatment) were dehydrated through an ethanol series, cleared in xylene, and embedded in paraffin for sectioning. Serial sections, cut at 7um with a rotary microtome (Leica RM 2265), were stained using haematoxylin and eosin (H&E), using an automated slide stainer (Leica Autostainer XL). Slides were examined using a Zeiss AxioImager A1 microscope, first at 100X magnification to obtain an overview of the gill tissue and to determine presence of parasites or large abnormalities. Then, secondly at 400X, gill lamellae were individually inspected to determine observable abnormalities and structural changes including epithelial lifting, telangieactasis (aneurysm), lamellar hyperplasia (distal=clubbing), fusion, basal hyperplasia, inflammation and other abnormalities. Eosinophilic granulocytes (EGCs) were manually identified and counted on every lamella, if present, for twelve histological sections of each gill, with a total of 72 gill sections investigated for each treatment.

STATISTICAL ANALYSIS

All enzymatic and protein data were analyzed for tank and sex effects using two-way ANOVA (treatment/tank, treatment/sex) for each period. When a significant difference was observed the Holm-Sidak post-hoc test was used to determine significant differences between groups. Two-way ANOVA was used to analyze data collected from EGCs counts (treatment/tank). Treatment/sex was not analyzed as all fish randomly selected for this aspect of the study were females or unknown sex. For all analyses, $P < 0.05$ was considered to be significant. Coefficients of variation were calculated for EGCs counts between

treatments using the calculation: Standard Deviation/Mean * 100 and reported as a percent.

RESULTS

Effect of sampling day was shown to not be significant ($P > 0.05$) and thus the samples were not influenced by the delay in sampling time of 50% of the tanks.

ENZYMATIC ANALYSES

No significant differences ($P > 0.05$) in enzymatic analyses of LDH, lactate, CS, and CCO were found between gill extracts of salmon exposed to normoxic and hypoxic conditions (Table 1). However, a significant increase ($P = 0.001$) in protein levels in gill extracts of salmon exposed to the hypoxic conditions was detected (Table 1).

Table 1: Enzymatic activity (LDH, LACT, CS, CCO) and protein (PROT) data of Atlantic salmon gill extracts following exposure to normoxic and hypoxic conditions.

	Normoxia (n=14)	Hypoxia (n=14)
PROT	7.82 ± 0.70	8.75 ± 0.65 *
LDH	0.0468 ± 0.0134	0.0522 ± 0.0154
LDH/mg Prot	1.201 ± 0.382	1.254 ± 0.382
LACT	0.275 ± 0.0898	0.304 ± 0.113
CS	0.499± 0.328	0.373 ± 0.344
CS / mg Prot	7.818± 0.700	8.748 ± 0.648
CCO	0.0198 ± 0.0131	0.0155 ± 0.0110
CCO/ mg Prot	0.00253 ± 0.00158	0.00173 ± 0.00113

PROT: proteins (mg/ml), LDH: lactate dehydrogenase (µmoles/min/ml), LACT: lactate (nmol/µL), CS: citrate synthase (µmoles/min/ml), CCO: cytochrome c oxidase (CCO activity/ml).
** significant difference*

GILL HISTOLOGY

No abnormalities were detected in gross morphological examination of gills from normoxic and hypoxic exposed fish. Eosinophilic granulocytes stained intensely eosinophilic and were located primarily in the non-lamellar portion of gill tissue (Figure 1), with rare occurrence of EGCs in actual gill lamella. Quantitative analysis of EGCs also revealed no significant differences between the two

treatment groups; P = 0.814 (Table 2). Frequency distribution did not reveal any differences in the distribution of EGC classes in both treatments (Figure 2).

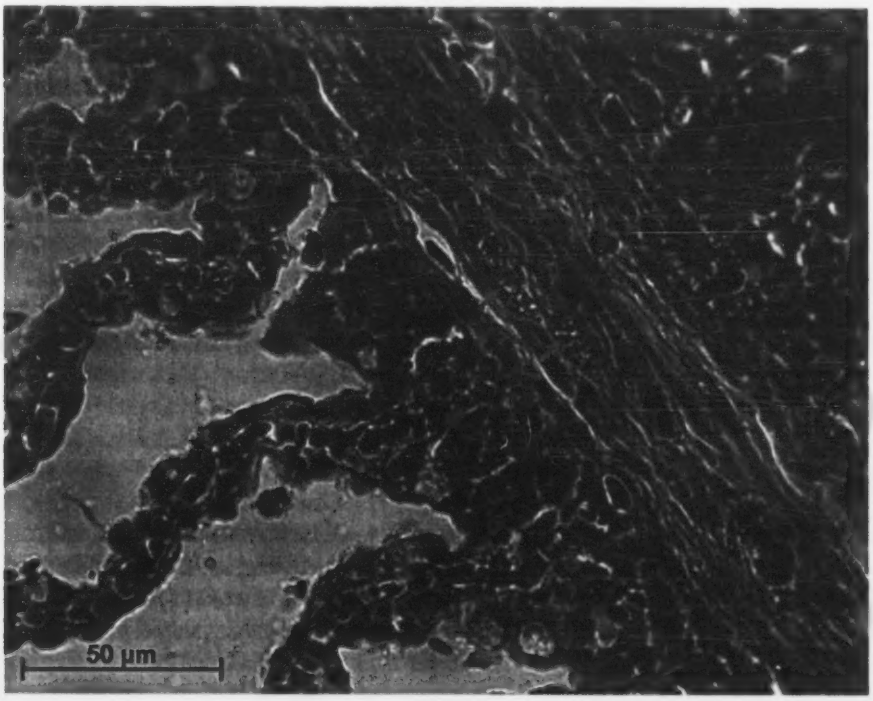

Figure 1: Eosinophilic granulocytes in Atlantic salmon gill (400X). Green arrows denote EGC location in non-lamellar portion of gill filament.

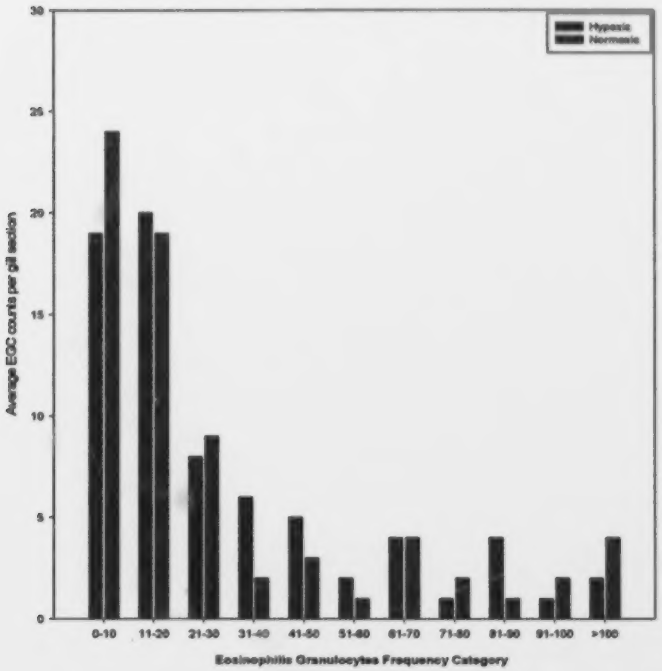

Figure 2: Frequency distribution of average EGC counts of hypoxia and normoxia exposed salmon.

Table 2: Quantitative assessment of eosinophilic granulocytes (EGCs) in gill filaments of Atlantic salmon exposed to normoxic and hypoxic conditions

	Hypoxic	Normoxic
Average EGCs per Filament	30.97	33.5
Total Filaments Counted	665	722

Coefficient of variation (CV) was high for mean counts of EGCs in both treatments (Table 3). Individual variation within treatments was large as demonstrated by CVs which ranged from 90.46% - 286.27%.

Table 3: Coefficient of variation of EGC mean counts in gills of Atlantic salmon exposed to normoxic and hypoxic conditions

Treatment	%
Hypoxia	52.50
Normoxia	54.00

DISCUSSION

Our findings reveal that exposure to intermittent hypoxia did not result in significant changes in tissue or enzymatic activity in gill of Atlantic salmon compared to their normoxic counterparts. Results did however indicate significantly increased protein concentrations in hypoxic over normoxic gills.

For Atlantic salmon, oxygen levels below the critical point of 6 mg L^{-1} are considered hypoxic (Davis 1975; DFO 2005; Mansour et al. 2008). We have used DO levels within the threshold cited earlier for the group exposed to intermittent hypoxia conditions. Lethal levels of low DO are not reached until 1mg L^{-1} (Brett and Groves 1979; Neill and Bryan 1991), and were not investigated in our study.

Intermittent hypoxia may have revealed lessened or fewer effects on gill tissues and enzymes than if fish were exposed to more prolonged episodes of hypoxia. Therefore, the full extent of potential change in function or immunity of gills exposed to intermittent hypoxia may not be seen. Fish may have had sufficient time for recovery and compensation for the effects of intermittent hypoxia, or the required thresholds in our experimental set up may not have been reached to induce changes in gill tissue and enzyme function. Burt et al. (2011) noted that avoidance swimming behaviour in Atlantic salmon was not observed in relation to water layers exhibiting low DO and proposed that the levels of DO recorded in that study may not be low enough to induce a behavioural change. This may also lend explanation to the lack of differences in gill enzymatic activity, as these DO concentrations too may not affect levels of observational change. In contrast however, Burt et al. (in press) observed changes in immunity indicators in intermittent hypoxia exposed salmon. Respiratory burst activity in leucocytes of head kidney was significantly higher in salmon of normoxic conditions over those exposed to intermittent hypoxia, suggesting immunity dysfunction in salmon exposed to intermittent periods of hypoxia.

Enzymatic analyses of gill tissue did not reveal significant differences in enzyme activity, similar to Webb et al. (2005) who found no significant differences in seasonal and annual levels of CCO in black bream (*Acanthopagrus butcheri*) gills sampled from various locations ranging from 5.3-10.6 mg L^{-1} DO. Cytochrome c oxidase is correlated well with oxygen consumption rates for different tissues and is found within mitochondria. Energy for the cell is provided by coupling electron transport through the cytochrome chain with the process of oxidative phosphorylation (Webb et al. 2005). Citrate synthase is an enzyme in the tricarboxylic acid cycle, a marker for mitochondrial volume and an indicator of aerobic respiration (Phillips et al. 2000; Cooper et al. 2002). In contrast to our results where no difference in CS activity was observed, Cooper et al. (2002) found a significant increase in CS in gill samples of the estuarine fish, *Leiostomus xanthurus*, exposed to 1 mg L^{-1} DO for 12 hrs as opposed to 3 mg L^{-1} and 11 mg L^{-1} treatments. In contrast to our results, increases in LDH content,

indicating the stimulation of anaerobic respiration have been reported in several species exposed to hypoxia (Cooper et al. 2002; Lewis and Driedzic 2010). However, in agreement to our study, Webb et al. (2005) found no significant differences in seasonal or annual LDH levels in black bream gills sampled from various locations ranging from 5.3 – 10.6 mg L^{-1} DO. Elevated LDH activity in gill tissues suggests that the aerobic catabolism of glycogen and glucose has shifted towards the anaerobic formation of lactate (Webb et al. 2005).

Of interest to the interpretation of our results, Lewis et al. (2007) determined that in the Amazonian cichlid (*Astronotus ocellatus*) lactate levels rapidly returned to pre-hypoxic levels (two hours post hypoxia exposure). It is most certainly a consideration that recuperation from the intermittent hypoxic episodes in the current study may have occurred in the hours prior to sampling, resulting in the elimination of any significant differences in gill tissue between the normoxic and hypoxic treatment groups in the enzyme activity tested.

In the present study, protein concentration was the only significantly different parameter between the two treatments, being increased in the hypoxia group. Protein synthesis, a generally energetically costly process, (Rolfe and Brown 1997) would intuitively be down-regulated in times of environmental stress, increasing survival time when subjected to sub-optimal environments (Guppy et al. 1994). Gill tissue, along with liver is a major source of protein synthesis (Lewis et al. 2007). Increased production of the proteins, myoglobin and haemoglobin, have been noted to be induced by hypoxia (Hoppeler and Vogt 2001), and substantially so in the liver, gills and brain (Fraser et al. 2006). In a recent study on Atlantic cod, Petersen & Gamperl (2011) reported an increased blood haemoglobin concentration of 10-15% following more than 6 weeks of hypoxia exposure (water O_2 content: 4.6 mg L^{-1} saturation). The higher concentration of protein synthesized in the hypoxic group could also be accounted for by a number of other possibilities. Similar to our findings, Soivio et al. (1981) found a slight increase in plasma proteins of rainbow trout when exposed to hypoxic conditions, and fluctuations in protein concentration in the recovery period, notably between 1-3 hours post hypoxia exposure. However, Lewis and Driedzic (2010) demonstrated a 40% reduction (non-significant) in protein synthesis in whole gill tissue of cunner (*Tautogolabrus adspersus*) exposed to acute hypoxia, albeit a protection of the proteins destined for gill mitochondria under these conditions was confirmed. Their proposed explanation, which may also shed light on the increased protein concentration in our study, was that defence of proteins in the mitochondria of gills ensures the continued synthesis of those proteins slated for epithelial and/or chloride cells necessary for any potential gill remodelling. It has also been proposed that proteins may potentially aid in quicker response of mitochondrial respiration in the gill upon reinstatement of more optimal environmental conditions (Sollid and Nilsson 2006; Lewis and Dreidzic 2010).

Furthermore, the increase in proteins detected in our study could also be a reflection of an increase in stress-related proteins (SRPs) resulting from the hypoxia stress response. The hypoxia stress response elicits the upregulation of several genes including: hypoxia-inducible factor alpha-1 (HIF-1α), heat shock cognate 70-kDA protein, erythropoietin, and beta and alpha globin genes (Guan et al. 2011). Sollid et al. (2006) demonstrated accumulation of HIF-1α protein during hypoxia, with the highest concentration detected after six hours of hypoxic exposure, however decreased to normoxic levels after 48 hours of exposure. These genes work together to make up the core signalling pathway of the hypoxia stress response which results in a series of biochemical and physiological changes allowing the animal a greater chance of survival under hypoxic conditions (Wu 2002; Sollid et al. 2006).

Exposure of animals to environmental stressors (e.g. hypoxia) to which they are not accustomed often results in the synthesis of stress related proteins during the recovery phase once the stressor is removed (Brooks and Storey 1993). Stress-related proteins, in heat-stressed eukaryotes, migrate from the nucleus to associate with other proteins to possibly aid in stabilizing nuclear material. However SRPs often are increased following, not during, a hypoxic event to promote cellular repair and preventing damage during reoxygenation (Brooks and Storey 1993). This concurs with our study, as salmon were sampled during the recovery phase, 14-38 hours following the last exposure to intermittent hypoxia.

No significant differences were found between treatments in the quantitative expression of EGCs. Levels of hypoxia may not have been low enough or adequately sustained to result in a histologically observed change in the gills or a change in the cellular immune response by an increase/decrease in EGC concentration. Eosinophilic granulocytes have previously been noted in gills of rainbow trout (Powell et al. 1990) and winter flounder (Murray et al. 2003). Powell et al. (1990) described EGCs in the capillary endothelia and free within the capillary lumen, and noted that EGC migration normally occurs within the microcirculation of the gill independent of infection, similar to the present observations. Also, Holland and Rowley (1998) found no significant differences in number of EGCs in gills of rainbow trout after long term handling and confinement stress (although differences were detected in those fish exposed to short term stress). Murray et al. (2003) identified EGCs in the non-lamellar portion of the gill filament and determined that the antimicrobial peptide (AMP), pleurocidin, was expressed by these cells. Antimicrobial peptides have been noted to be important in the immunoresponse of many species (Hancock and Scott 2000), and in epithelial surfaces act in the mucous as a barrier to microbial invasion from the environment, and may circulate in response to injury or inflammation (Murray et al. 2003). Our observation of EGCs throughout gills in all groups, regardless of environmental stress further confirms Powell's conclusions, however does not appear to arise as an immuno-response to environmental stressors as proposed by Murray et al. (2003) and Holland and

Rowley (1998). This study indicates that there may be a population of EGCs that are resident in the gills of salmon, and these cells may act as a primary defence system, regardless of environmental oxygen parameters. It would be interesting to determine if any changes in the gill tissue or cellular structure would be apparent if salmon were exposed to longer or more extreme incidents of intermittent hypoxia. Given, these results, one may be able to interpret that salmon gills are resilient to intermittent hypoxia in the conditions described in this study and animals are able to recover from these events with minimal negative outcomes to gill tissue.

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